Supporting information:

Bacterial Cytological Profiling Reveals the Mechanism of Action of Anticancer Metal Complexes

Yang Sun, David Heidary, Zhihui Zhang, Christopher Richards, and Edith Glazer *

 Department of Chemistry, University of Kentucky, Lexington, KY 40506, United States
* Email: ec.glazer@uky.edu

Contents

Calculation of the ratio of DNA and RNA nucleotides to metal based on data reported by DeRose et al.[1]:

The platinum content measured after 12 hours treatment of 100 µM cisplatin was used for this calculation.

$Pt / DNA\ nt = 60.2 \times 10^{-5}$

$$DNA\ nt / Pt = \frac{1}{60.2 \times 10^{-5}} = 1661:1$$

$Pt / RNA\ nt = 15.7 \times 10^{-5}$

$$RNA\ nt / Pt = \frac{1}{15.7 \times 10^{-5}} = 6369:1$$

Calculation of the ratio of DNA and RNA nucleotides to metal based on data reported from an IVTT assay[2] (at the IC_{50}):

DNA bp / Ru = 570:1

DNA nt / Ru = 1140:1

DNA bp / Pt = 300:1

DNA nt / Pt = 600:1

RNA nt / Ru = 610:1

RNA nt / Pt = 820:1

Calculation of the ratio of DNA and RNA nucleotides to metal at the IC_{50} to Dendra2 production inhibition based on data collected in AAS:

The DNA and RNA nt / mc ratio collected by AAS at 20µM is as follows:

DNA nt / Ru = 2000:1

DNA nt / Pt = 3000:1

RNA nt / Ru = 3800:1

RNA nt / Pt = 4700:1

Assuming the uptake is linearly proportional to the dosed concentration, the IC_{50} value to inhibit Dendra2 production in *E. coli* is 77 µM for compound **1** and 85 µM for cisplatin. Thus, a correction coefficient can be calculated to adjust the metal content to what would be obtained at the higher compound dose:

$$\text{Compound } \mathbf{1}: \frac{77}{20} = 3.8$$

$$\text{Cisplatin}: \frac{85}{20} = 4.3$$

Then, at the IC_{50} to inhibit Dendra2 production, the theoretical metal to DNA or RNA nucleotides to metal ratio can be calculated as follows:

$$\text{DNA nt / Ru} = \frac{2000}{3.8} = 520 : 1$$

$$\text{RNA nt / Ru} = \frac{3800}{3.8} = 1000 : 1$$

$$\text{DNA nt / Pt} = \frac{3000}{4.3} = 700 : 1$$

$$\text{RNA nt / Pt} = \frac{4700}{4.3} = 1090 : 1$$

The average molecular weight values used in calculation for DNA base pairs and RNA nucleotides are 665 g/mol and 340 g/mol.

Table S1. Cellular metal content with different nucleic acids measured by AAS.

	E. coli		HL60	
	Percentage of metal bound with genomic DNA[a]	Percentage of metal bound with total RNA[b]	Percentage of metal bound with genomic DNA[a]	Percentage of metal bound with total RNA[b]
1 light	1.3% ± 0.1%	0.5% ± 0.1%	1.3% ± 0.1%	2.0% ± 0.3%
1 dark	[c]	[c]	[c]	[c]
cisplatin	1.0% ± 0.1%	0.7% ± 0.1%	1.1% ± 0.1%	1.5% ± 0.2%

[a, b]The percentage of metal bound with genomic DNA or total RNA was calculated as follows:

$$\text{Percentage of metal bound with DNA (RNA)} = \frac{\text{Metal measured in DNA (RNA) sample (μmol)}}{\text{Metal in DNA sample + cell sample (μmol)}}$$

[c] Ruthenium level in DNA and RNA samples were under the detection limit (<2ppb).
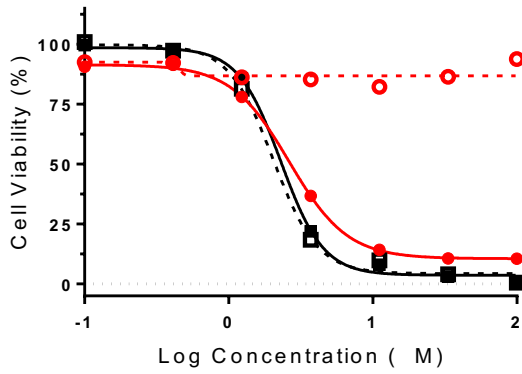
Figure S1. Cytotoxicity dose response of complex **1** (red lines) and cisplatin (black lines) in *E. coli* following photoirradiation (solid lines, filled circles) and dark condition (dashed lines, open circles).
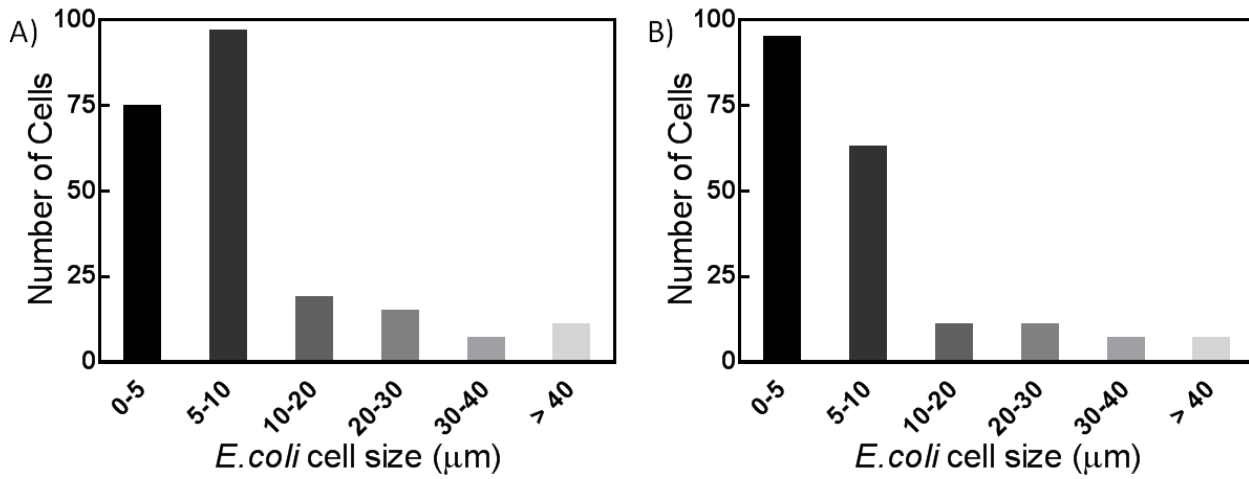

Figure S2. Size distribution histograms of *E. coli* filaments with treatment of A) rifampicin; and B) tetracycline. *E. coli* cells were treated with 3 µM of rifampicin and 48 µM of tetracycline (10x IC_{50}) for 6 hours.
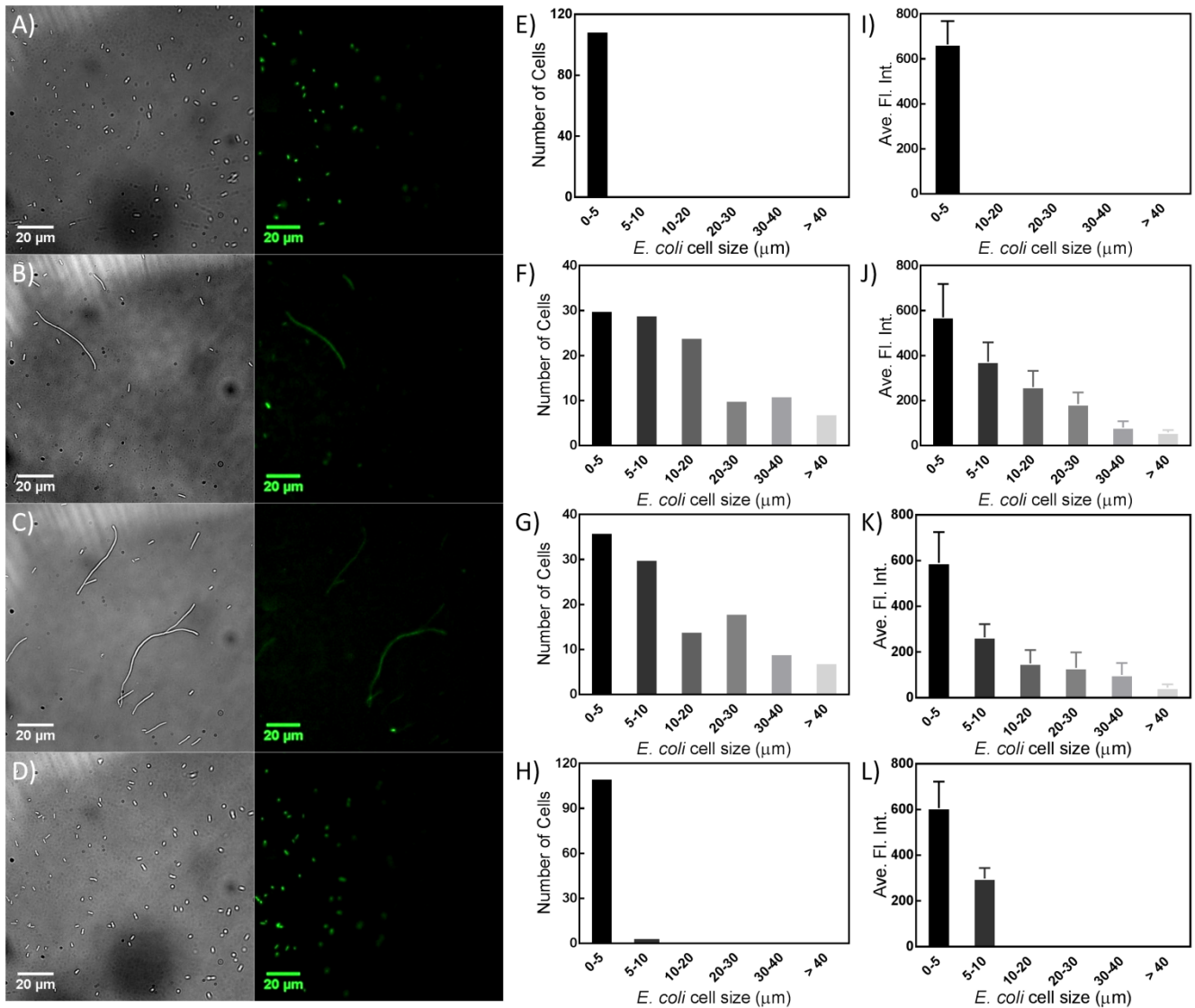
Figure S3. Complex **1** induces filamentous growth and decreased protein production in *E. coli*. A) - D) Bright field and fluorescent imaging of *E. coli* cells under different conditions; E) - H) Size distribution histograms of *E. coli* cells under different treatments; I)- L) Histograms of average fluorescence intensity (Ave. Fl. Int.) correlating to cell size with different treatments. Top through bottom panels: N. C. control, cisplatin, compound **1** with light, and compound 1 without light. Cells were treated with 40 μM each compound for 6 hours before imaging.
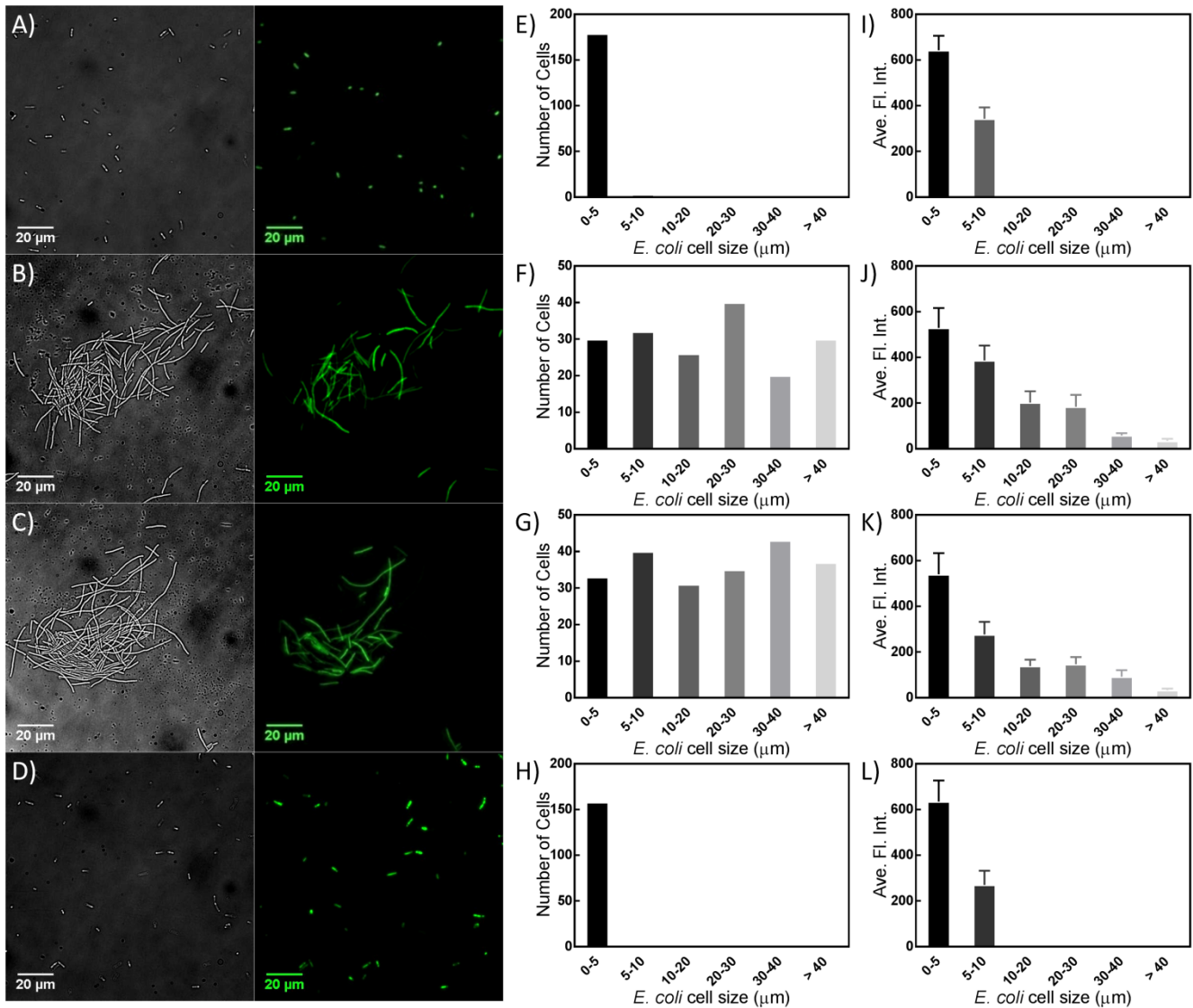
Figure S4. Complex **1** induces filamentous growth and decreased protein production in *E. coli*. A) - D) Bright field and fluorescent imaging of *E. coli* cells under different conditions; E) - H) Size distribution histograms of *E. coli* cells under different treatments; I)- L) Histograms of average fluorescence intensity (Ave. Fl. Int.) correlating to cell size with different treatments. Top through bottom panels: N. C. control, cisplatin, compound **1**with light, and compound **1**without light. Cells were treated with 40 μM each compound for 16 hours before imaging.
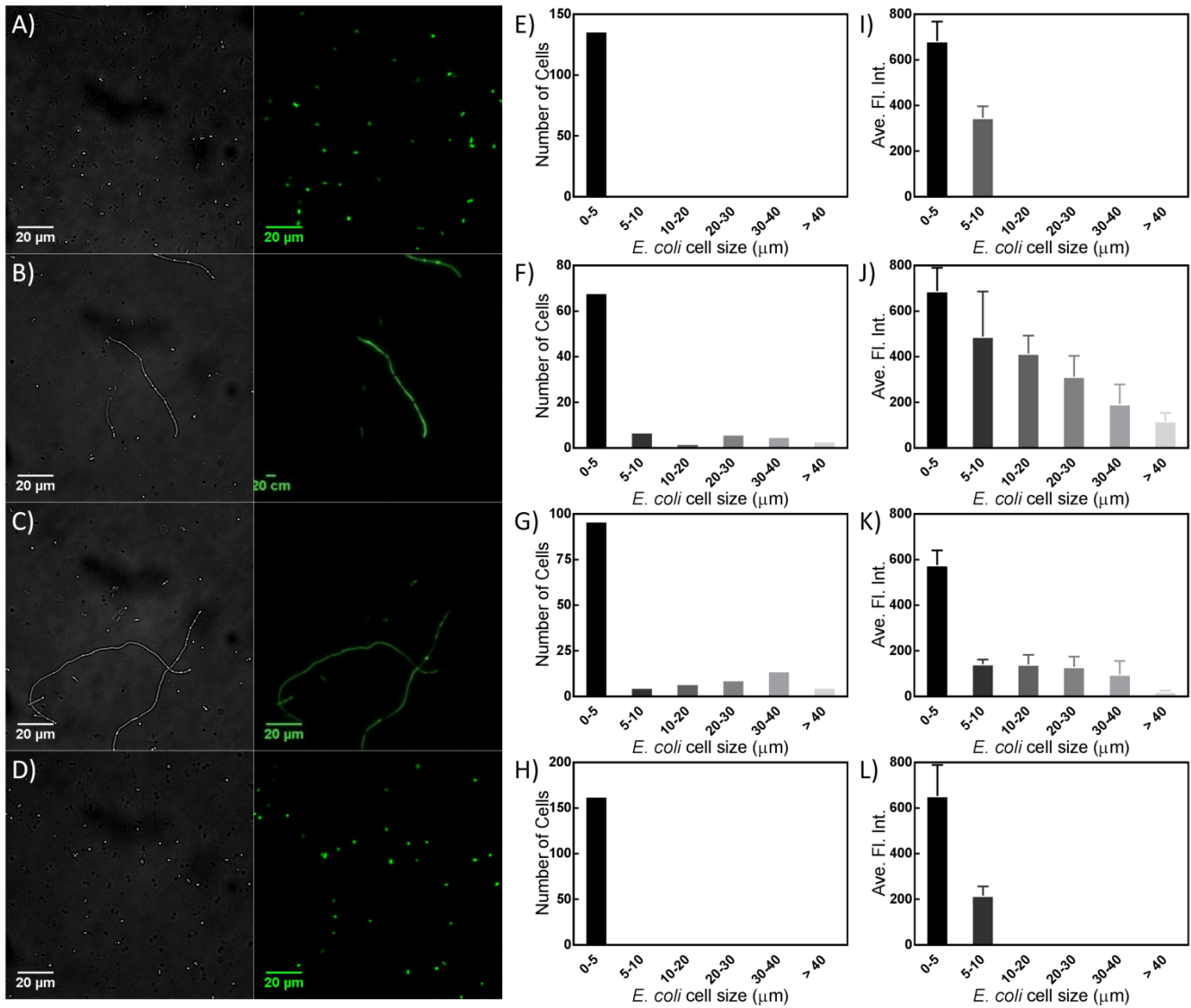
Figure S5. Complex **1** induces filamentous growth and decreased protein production in *E. coli*. A) - D) Bright field and fluorescent imaging of *E. coli* cells under different conditions; E) - H) Size distribution histograms of *E. coli* cells under different treatments; I)- L) Histograms of average fluorescence intensity (Ave. Fl. Int.) correlating to cell size with different treatments. Top through bottom panels: N. C. control, cisplatin, compound **1**with light, and compound **1**without light. Cells were treated with 100 μM each compound for 16 hours before imaging.
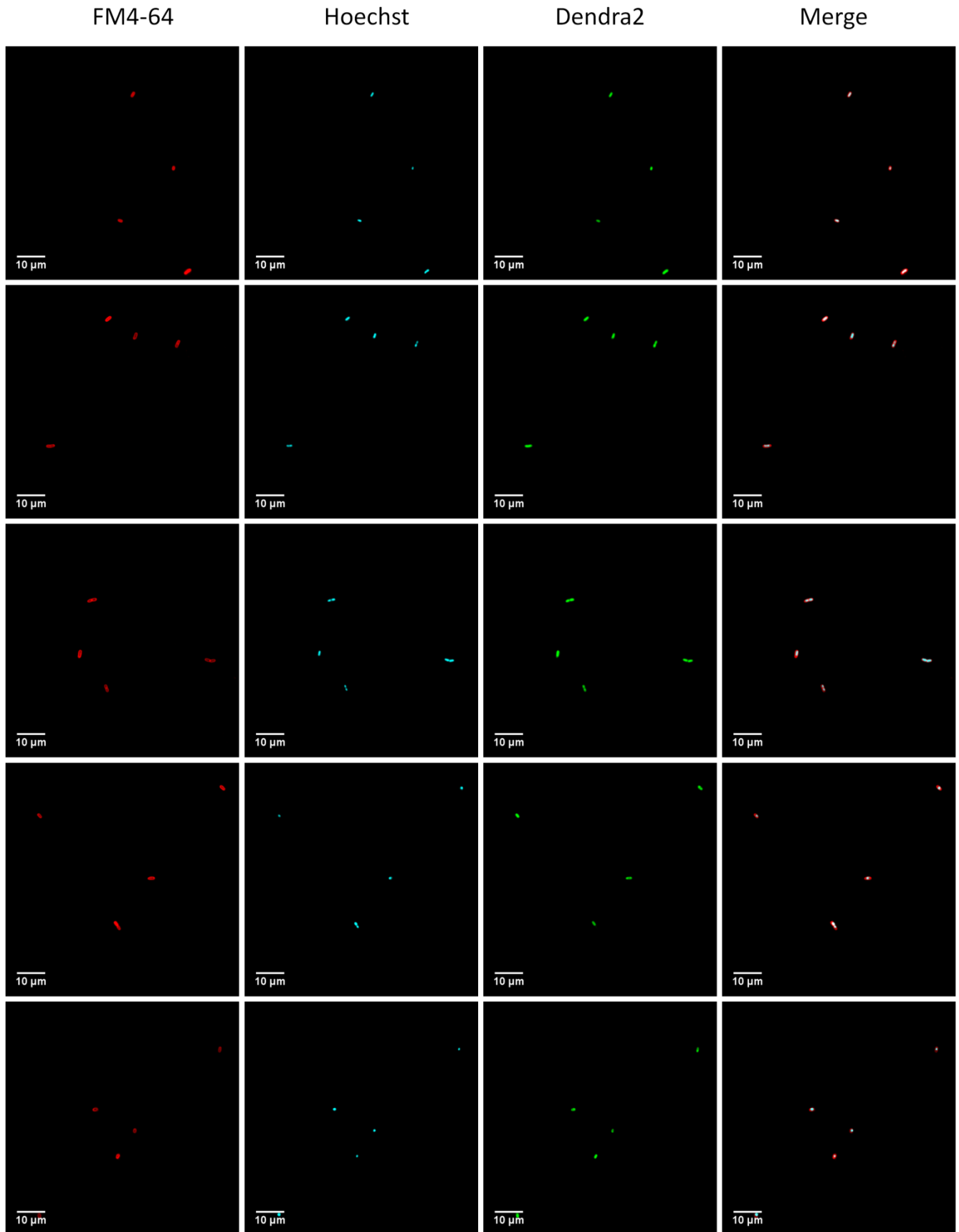
| FM4-64 | Hoechst | Dendra2 | Merge |

Figure S6. Supplemental fluorescent imaging of N. C. control. The merge is the combination of the Hoechst and FM4-64 membrane stain emission data.

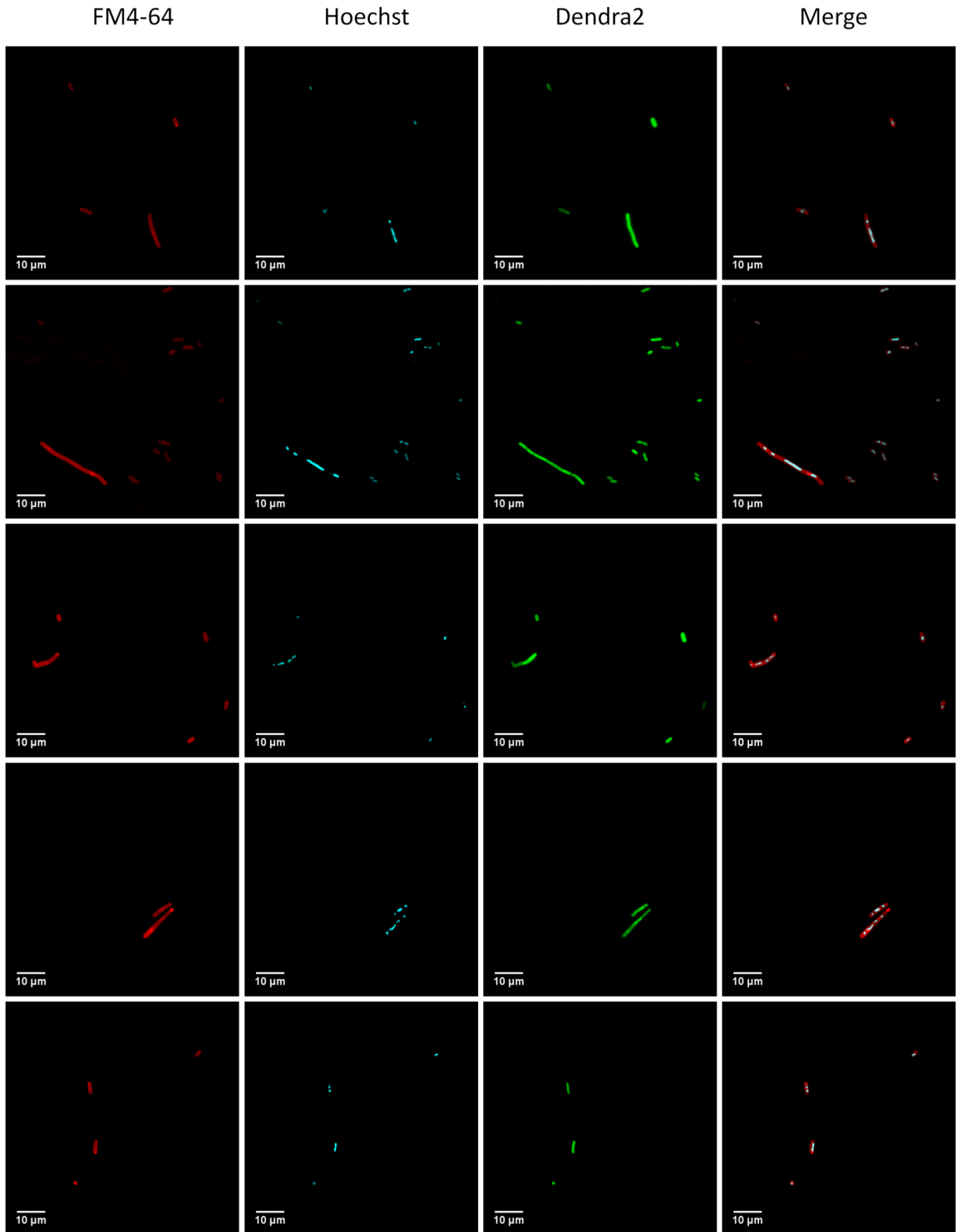| FM4-64	Hoechst	Dendra2	Merge

Figure S7. Supplemental fluorescent imaging of cisplatin. Cells were treated MIC for 6 hours before imaging. The merge is the combination of the Hoechst and FM4-64 membrane stain emission data.

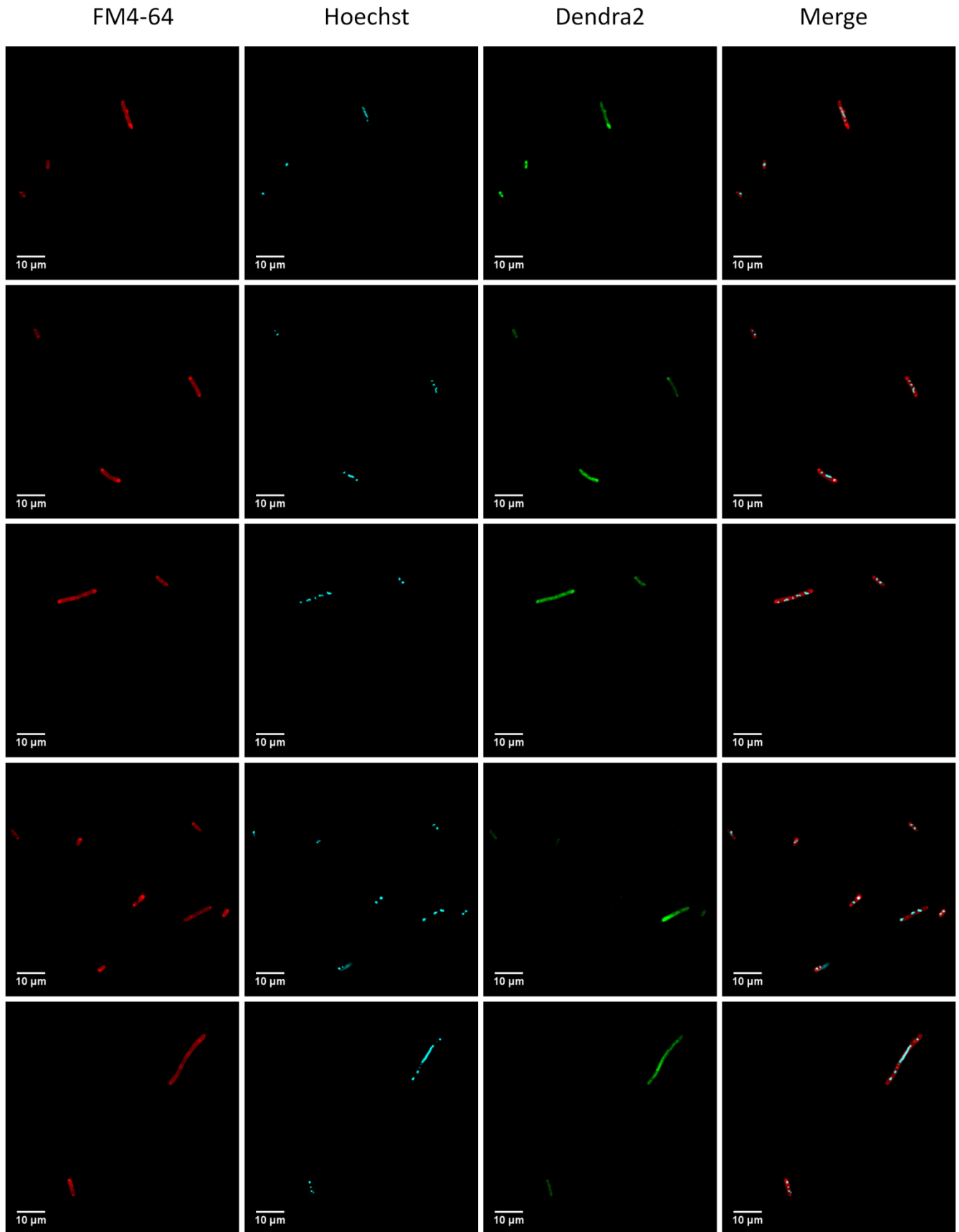| FM4-64	Hoechst	Dendra2	Merge

Figure S8. Supplemental fluorescent imaging of compound **1** with light. Cells were treated MIC for 6 hours before imaging. The merge is the combination of the Hoechst and FM4-64 membrane stain emission data.
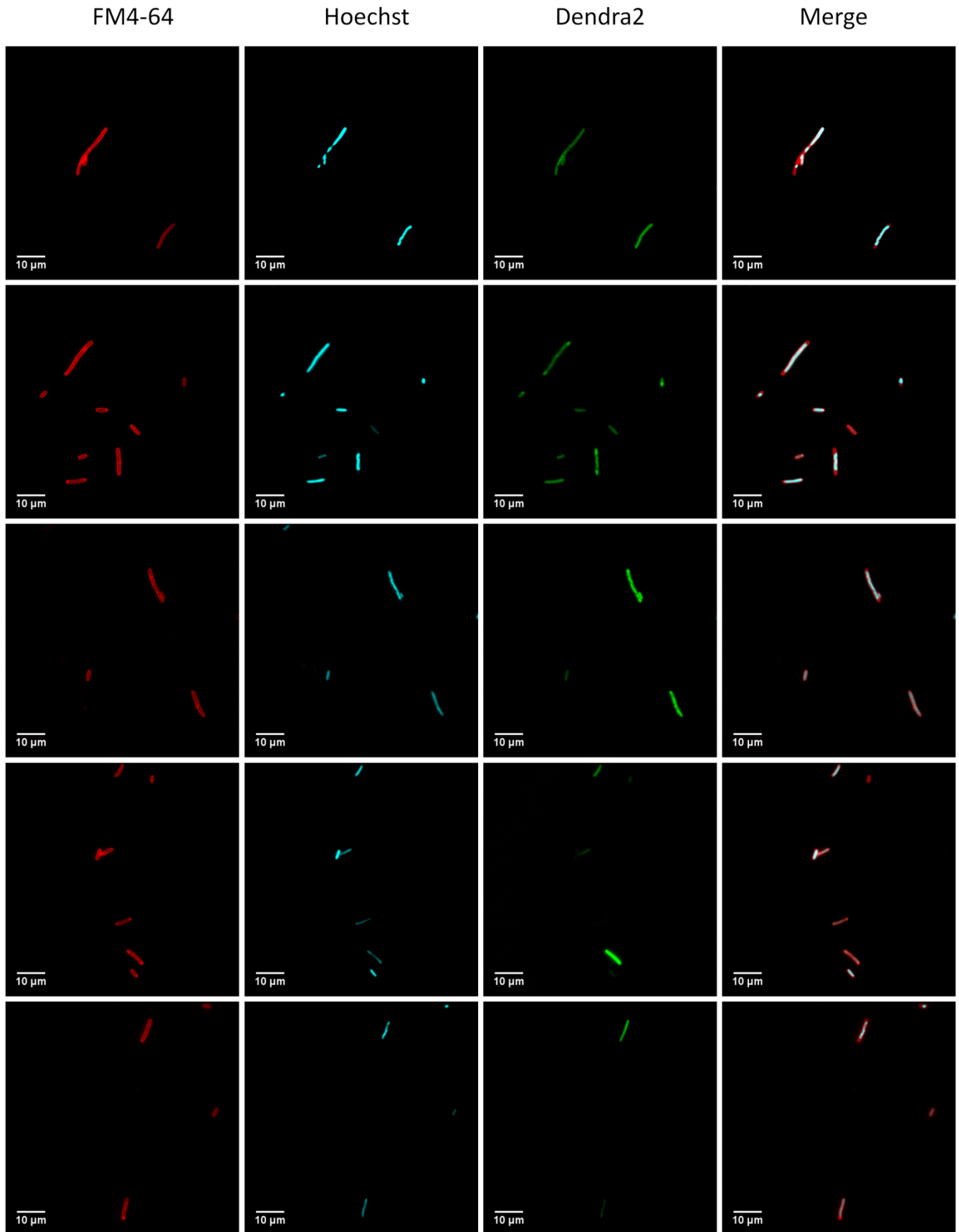
FM4-64　　　　　　Hoechst　　　　　　Dendra2　　　　　　Merge

Figure S9. Supplemental fluorescent imaging of rifampicin. Cells were treated 10x MIC for 6 hours before imaging. The merge is the combination of the Hoechst and FM4-64 membrane stain emission data.

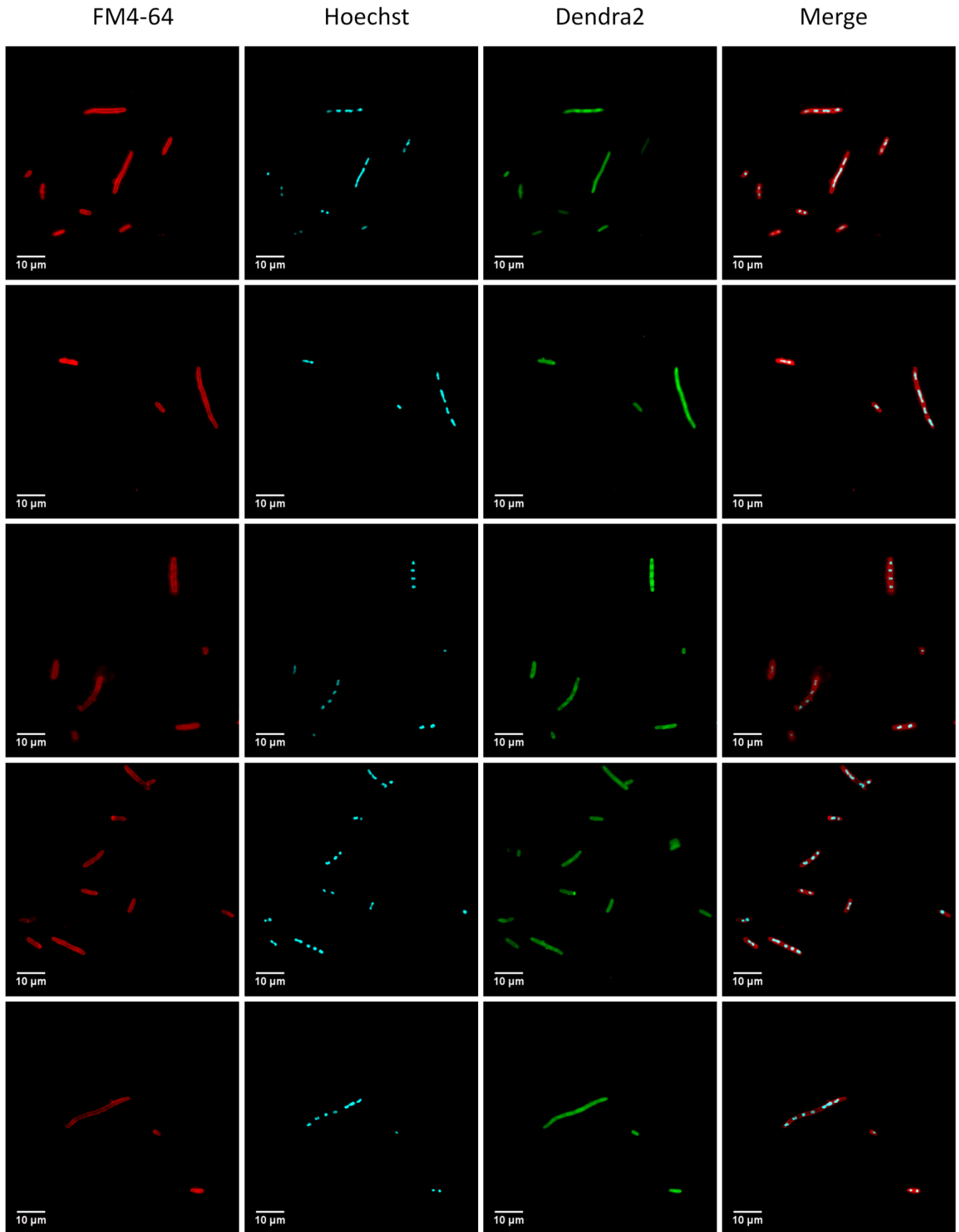| FM4-64	Hoechst	Dendra2	Merge

Figure S10. Supplemental fluorescent imaging of tetracycline. Cells were treated MIC for 6 hours before imaging. The merge is the combination of the Hoechst and FM4-64 membrane stain emission data.
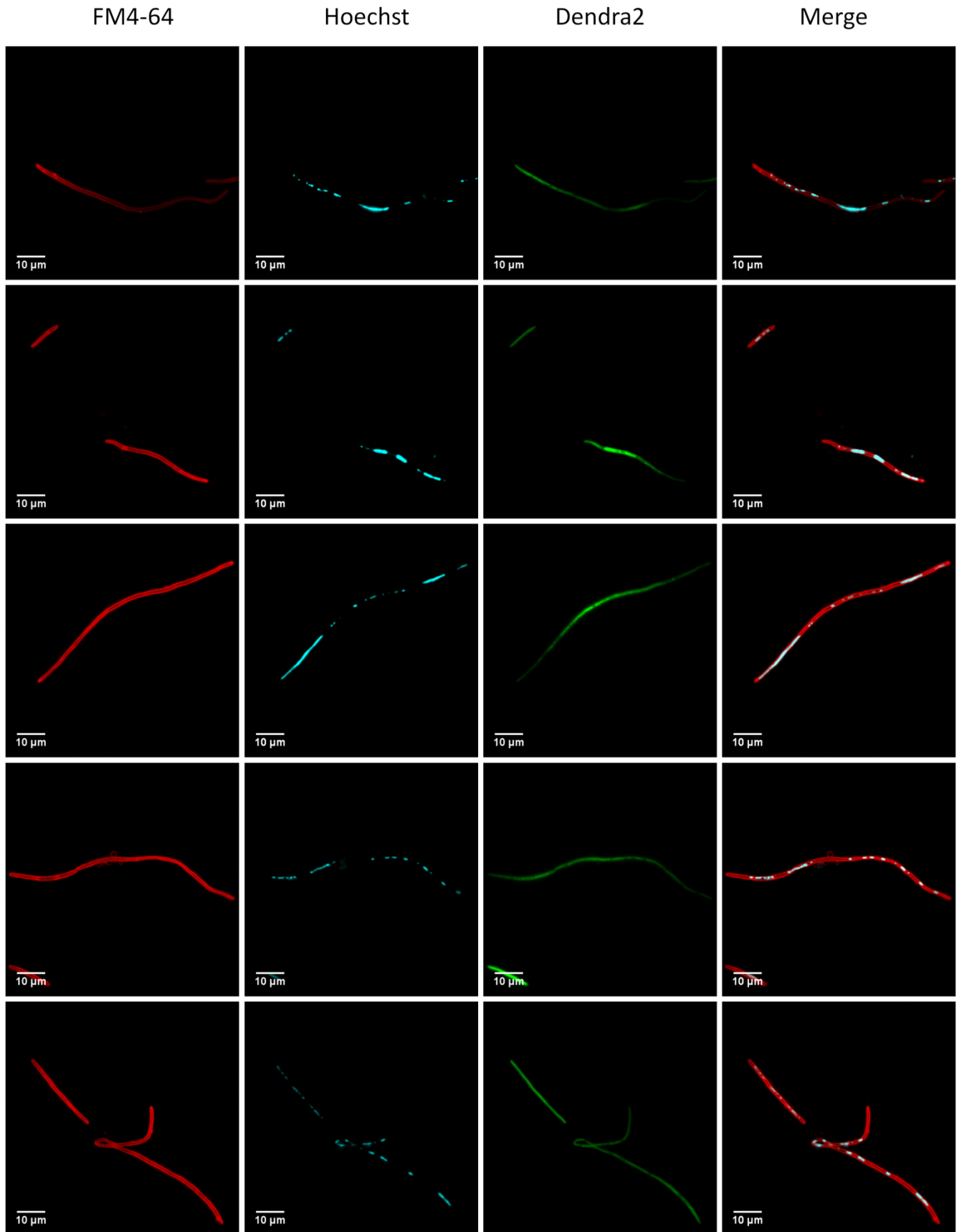
Figure S11. Supplemental fluorescent imaging of nalidixic acid. Cells were treated MIC for 6 hours before imaging. The merge is the combination of the Hoechst and FM4-64 membrane stain emission data.
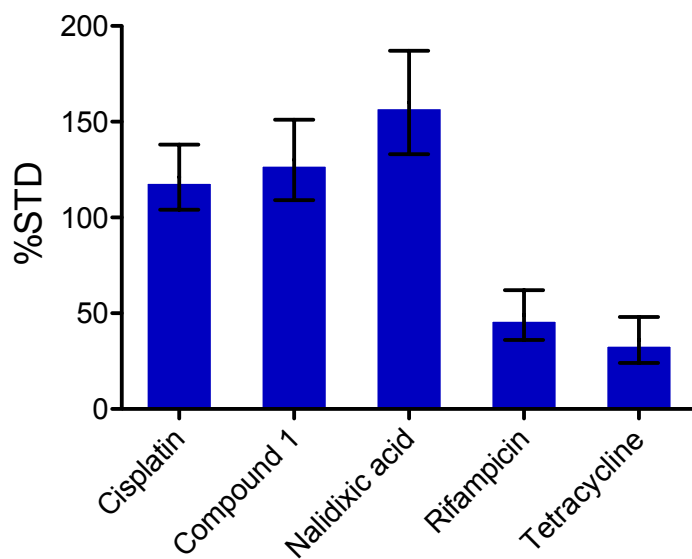
Figure S12. Standard deviations in DNA fragment size for the various compound treatments.

The %STD of DNA fragments was defined and calculated as follows:

$$\%STD\ (DNA\ pieces\ size) = \frac{\text{Standard deviation of sizes of DNA pieces}}{\text{Average of sizes of DNA pieces}}$$
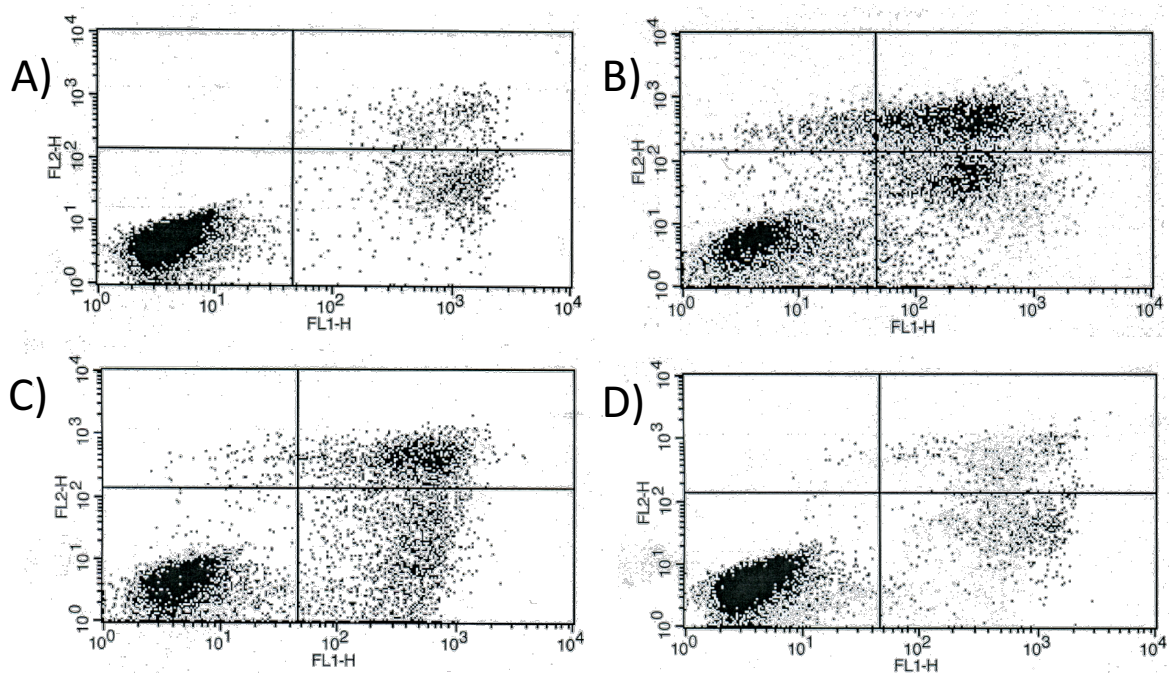
Figure S13. Flow cytometry analysis by FITC/Annexin-V and PI of apoptosis in HL60 cells. FITC-Annexin V (FL1-H) was used in combination with propidium iodide (FL2-H). A) N. C. control; B) cis-platin; C) compound **1** with light irradiation; D) compound **1** in dark. HL60 cells were treated with 20 μM compounds for 24 hours.
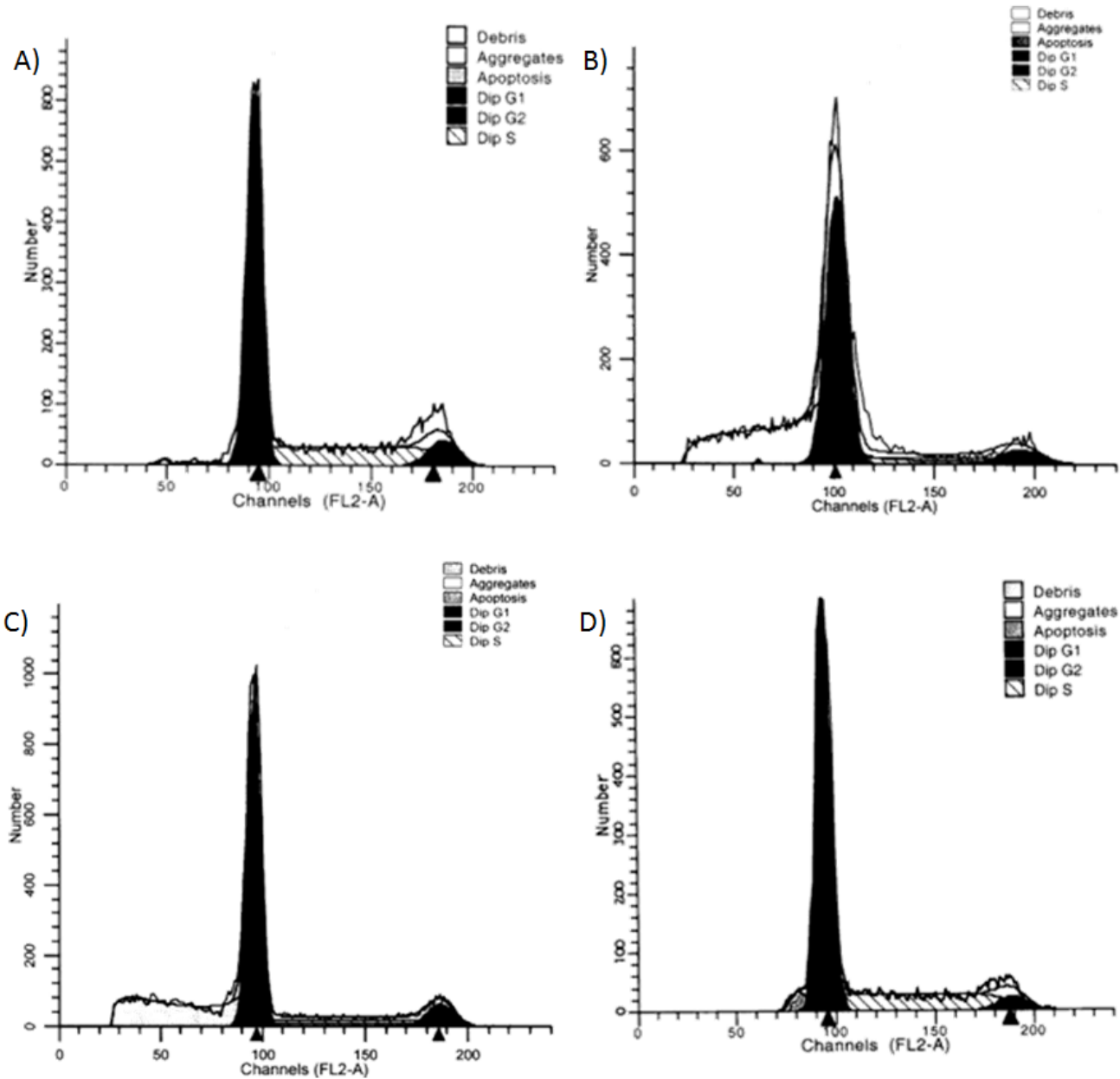
Figure S14. Flow cytometry analysis by propidium iodide of cell cycle arrest in HL60 cells. A) N. C. control; B) cisplatin; C) compound **1** with light irradiation; D) compound **1** in dark.HL60 cells were treated with compounds for 24 hours.

References:

1.   Hostetter, A. A.; Osborn, M. F.; DeRose, V. J., RNA-Pt adducts following cisplatin treatment of Saccharomyces cerevisiae. *ACS Chem Biol* **2012,** *7* (1), 218-25.
2.   Heidary, D. K.; Glazer, E. C., A light-activated metal complex targets both DNA and RNA in a fluorescent in vitro transcription and translation assay. *Chembiochem* **2014,** *15* (4), 507-11.